Toll-Like Receptors and Danger Signaling in Kidney Injury

Hans-Joachim Anders

Department of Nephrology, University of Munich, Munich, Germany

ABSTRACT

Why does renal inflammation appear among many of the so-called noninflammatory kidney diseases? Toll-like receptor research provides a surprising answer because activation of the innate immune system involves pathogen-derived as well as nonpathogen-derived immunostimulatory molecules; thus, metabolic, hemodynamic, toxic, or autoimmune forms of tissue damage all can trigger an innate inflammatory response. Because receptor activation is unable to eliminate the underlying drivers of these nonpathogen diseases, it becomes instead a maladaptive pathogenic mechanism that aggravates renal damage. Genetic variants in danger-signaling genes of the innate immune system can also affect individual risk for insufficient pathogen control or exaggerated nonpathogen-related tissue pathology. The evolving concept of danger signaling provides a general mechanism for kidney injury.

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Whereas inflammation is anticipated in diseases such as glomerulonephritis, renal vasculitis, and primary and secondary interstitial nephritis, varying degrees of renal inflammation also appear in a multitude of other kidney diseases generally considered noninflammatory, such as diabetic glomerulosclerosis, the nephropathy of aging, renal atherosclerosis, Alport nephropathy, and polycystic kidney disease, to mention a few.[1–6] Infectious, toxic, metabolic, ischemic, traumatic, and genetic causes of kidney injury all share an unexpected component of tissue inflammation. How does this happen?

I address this question by making three elementary observations. First, tissue damage producing renal inflammation is the price we pay for infectious pathogen control. Second, nonpathogen causes of tissue damage trigger inflammation just like pathogens because some intracellular molecules that are released during renal cell death and other extracellular molecules ligate the same danger sensors of the innate immune system, which is referred to as *danger signaling*. Third, polymorphisms in associated genes modulate genetic risk for immunopathology in kidney diseases.

Tissue damage generally threatens the integrity and function of delicate anatomic structures independent of causative factors. Kidneys respond to environmental perturbagens by activating their own defense mechanisms such as cellular proliferation[7,8] and the release of complement components,[9] antimicrobial defensins,[10] or matrix components into extracellular spaces.[11] In addition, kidneys engage extrinsic help for controlling danger by sending dendritic cells to regional lymph nodes[12] or releasing cytokines and chemokines that alert and recruit the professional danger controllers of the immune system.[4,13,14] The early sequential influx of neutrophils, macrophages, and T cells to the site of danger amplifies the risk for nonspecific inflammation, which is sometimes followed later by antigen-specific injury.[11,15] This conventional model of immune activation may apply to acute infections in the kidney, but is it helpful in understanding inflammation in more common, nonpathogen types of acute and chronic kidney disease? Addressing this question requires answering another one first. What are the sensors that recognize pathogens as well as nonpathogen types of danger?

IMMUNOPATHOLOGY IN PATHOGEN CONTROL

The discovery of the Toll-like receptors (TLRs) as one class of innate pathogen sensors has attracted tremendous interest. TLRs on the cell surface (TLR1/2/4/5/6) or in intracellular endosomes (TLR3/7/8/9) recognize the entire spectrum of potential pathogens by ligating so-called pathogen-associated molecular patterns, such as LPS (TLR4), lipopeptides (TLR1/2/6), and viral/bacterial nucleic acids (TLR3/7/8/9). Additional pathogen sensors lo-

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Correspondence: Prof. Dr. Hans-Joachim Anders, Medizinische Poliklinik der LMU, Pettenkoferstrasse 8a, 80336 München, Germany. Phone: ++49-89-218075855; Fax: ++49-89-218075860; E-mail: hjanders@med.uni-muenchen.de

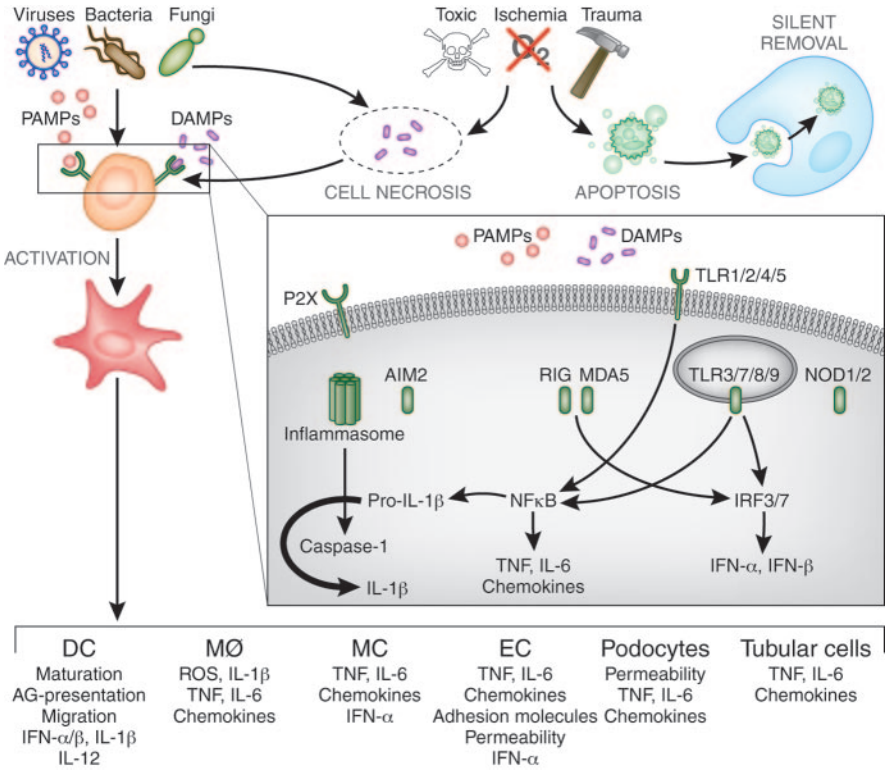

Figure 1. Pathogens and cell necrosis alert innate immunity. All classes of pathogens release pathogen-associated molecular patterns that can activate TLRs on the cell surface or in intracellular endosomes. TLR activation induces the expression of pro-IL-1β, NF-κB–dependent cytokines and chemokines, and IFN-α and IFN-β, the three dominant cytokine classes of innate immunity. NOD-like receptors and RIG helicases convert the recognition nucleic acids into cytokine release. Inflammasome-related sensors activate caspase 1, a necessary step for the secretion of IL-1β. Activation of such sensors has additional cell type–specific effects (*e.g.*, in dendritic cells [DC] or macrophages [MØ], mesangial cells [MC],[35] glomerular endothelial cells [EC],[36] or podocytes.[37,38] Cell necrosis can trigger identical effects because some intracellular molecules can act as DAMPs on the same receptors. Apoptotic cell death and rapid clearance by phagocytes avoids unnecessary immune activation.

calize within the intracellular cytosol, such as the RIG-like helicases that detect viral nucleic acids, the NOD-like receptors that detect bacterial peptides, and the inflammasomes that integrate several triggers for the secretion of IL-1β (Figure 1).

Renal cells classically use these TLRs to signal kidney infection, albeit in a cell type–specific manner.[15,16] For example, LPS-derived from uropathogenic *Escherichia coli* ligates TLR4 on tubular epithelial cells and intrarenal dendritic cells, a process triggering the release of neutrophil chemokines, which ensures pathogen clearance to avoid urosepsis after infectious pyelonephritis.[17,18] Hence, pathogen recognition and pathogen-directed immune responses are necessary to control infections, but TLR4-induced innate immunity is also responsible for renal abscess formation and tissue destruction in infectious pyelonephritis.[17] TLR9 activity also aggravates IgA nephropathy in mice housed in conventional caging compared with caging in pathogen-free environments, suggesting that native microbial flora heightens the sensitivity of the innate immune system.[19] It is a central paradigm that tissue damage (ranging from negligible to extensive necrosis or scarring) is the price we pay for rapid and effective infection control because the cost–benefit ratio of pathogen control *versus* unwanted immunopathology favors rigorous host defense during evolution.

NONINFECTIOUS TISSUE DAMAGE SIGNALS DANGER LIKE THE INFECTIOUS PATHOGENS

Many clinical settings do not fit into the classical pathogen- or antigen-driven paradigm of immune activation, as pointed out by Matzinger[20] long before danger signaling emerged as a concept. As one example, gouty attacks and bacterial arthritis with massive local inflammation are often clinically indistinguishable forms of sudden-onset monoarthritis. Only recently has it become clear that bacterial products and uric acid crystals both activate the NLRP3 inflammasome, leading to the immediate secretion of preformed IL-1β.[21] Uric acid is also released by dying cells during tissue damage, a process that contributes to innate immune activation during cell necrosis.[22] A number of other intracellular damage–associated molecular patterns (DAMPs) also ligate TLRs and other innate immune receptors (Table 1). For example, high-mobility group box 1 is a nuclear protein that, once released by necrotic cells, can ligate TLR4 and shuttle immunostimulatory nucleic acids to intracellular nucleic acid receptors. Mice lacking high-mobility group box 1 are protected from postischemic acute renal failure because initial tubular cell necrosis no longer triggers postischemic renal inflammation and subsequent tubular damage.[23]

Binding to TLRs is a central element of danger signaling because TLR activation is required to induce pro-IL-1β, NF-κB–dependent cytokines, and type I IFNs, the three dominant cytokine classes underlying innate immunity (Figure 1). By contrast, apoptotic cell death avoids DAMP release, and phagocytic uptake of apoptotic cells instead elicits anti-inflammatory effects, a mechanism contributing to tissue homeostasis under physiologic conditions. Genetic defects that impair apoptotic cell death or the proper clearance of apoptotic debris can turn the "silent way of death" into inflammation and autoimmunity. This is because late apoptotic cells undergo secondary necrosis, a process exposing nuclear autoantigens and intracellular DAMPs to the innate immune system.

In lupus, for example, the potential dual function of endogenous RNA and DNA

Table 1. Self-molecules that activate innate immunity

DAMP	Receptor
Adenosine	A1/A2A/A2B/A3
ATP	P1/P2X/P2Y/NLRP3
Biglycan	TLR2, TLR4
Cathepsin-B	NLRP3
CpG DNA (hypomethylated)	TLR9
Defensins	TLR4
DNA-nucleosomes-IgG	TLR9 (FcR/BCR)
dsDNA	AIM2, ?
Fibrinogen	TLR4
Heat-shock proteins	TLR2, TLR4
Heparan sulphate	TLR4
HMGB1	TLR2, TLR4, RAGE, RIG
Hyaluronates	TLR2, TLR4
U1snRNP-IgG	TLR7 (FcR/BCR)
Uric acid crystals	NLRP3

AIM, absent in melanoma; BCR, B cell receptor; HMGB1, high-mobility group box 1; NLRP, Nacht domain–, leucine-rich repeat–, and PYD-containing protein.

moieties are most illuminating.[24,25] The ribonucleoprotein U1snRNP acts as an autoadjuvant by activating TLR7, which synergizes with its role as a lupus autoantigen to trigger RNA-specific T and B cell autoimmunity. Similarly, hypomethylated chromatin acts as an autoadjuvant by activating TLR9, which synergizes with its role as an autoantigen inducing DNA-specific T and B cell autoimmunity in lupus nephritis. The misinterpretation of endogenous nucleic acids as autoantigens and autoadjuvants can no longer be understood as a tissue-derived danger signal that triggers danger control. This rather represents an example of maladaptive or inappropriate immunity whereby obfuscation of the distinction between self and foreign nucleic acids results in a "pseudoantiviral" immune response, causing autoimmunity, lupus, and nephritis.[24,25]

Pathogens and necrotic cells during infection both send independent danger signals to alert the immune system for host defense. Necrotic cells alone in nonpathogen diseases also trigger inflammation through the release of DAMPs. This mechanism has a dual effect; although cell necrosis stimulates the recruitment of phagocytes for the removal of cellular debris, the ensuing renal inflammation usually cannot resolve the underlying nonpathogen basis that stimulated the cell death originally. For example, complement activation or immune cell infiltrates in renal tissue are typically unable to control threats external to the kidney, such as hyperglycemia, nephrotoxin exposure, and circulating immune complex disease, as well as renal artery stenosis, embolism, shock, or other reasons for renal ischemia. However, this intrarenal inflammation promotes unwanted immunopathology. As such, danger signals that are evolutionarily selected for control of infection often turn into a maladaptive mechanism whereby inappropriate intrarenal inflammation perpetuates damage and scarring.

DANGER SIGNALING AND THE GENETIC RISK FOR KIDNEY DISEASE

Measured control of immune activation remains an Achilles' heel, because it needs to be sufficient and sustained for appropriate host defense but not too strong or persistent to avoid unwanted tissue pathology. It is widely known to clinicians that both insufficient and robust immune responses cause morbidity and mortality, but which factors regulate the extent and duration of TLR signaling? Do genetic polymorphisms in relevant genes determine risk for either insufficient or persistent activation of innate immunity?

For example, human polymorphisms in the genes encoding *TLR9* and the central TLR signaling adaptor, *MyD88*, associate with progression of IgA nephropathy.[19] Humans lacking *MyD88* are also prone to life-threatening pyogenic infections,[26] and humans with mutant TLR signaling inhibitors are at risk for chronic airway inflammation and asthma.[27] TLR4 activation in mice also mediates *MyD88*-dependent kidney inflammation after exposure to toxins,[28] whereas *MyD88*-deficient mice are protected from immunopathology in several kidney diseases, including experimental glomerulonephritis, postischemic acute renal failure, and kidney allograft dysfunction.[29–31] *Vice versa*, mice that lack the TLR signaling inhibitor SIGIRR develop aggravated immunopathology in these models.[32–34] Obviously, innate danger control is tightly regulated and genetic variants in TLR signaling modulate risk for renal progression. Now we need to determine in a similar manner whether human gene variants modify tissue pathology in these and other kidney diseases.

CONCLUSIONS

Some inflammation accompanies nonpathogen injury to the kidney because damaged renal tissues activate innate immunity by releasing immunostimulatory molecules. TLRs on or in kidney cells transduce the recognition of such danger signals into the secretion of IL-1β, type I IFNs, and NF-κB–dependent cytokines and chemokines. This innate immune response induces leukocyte infiltrates and immune-mediated tissue injury in most types of kidney disease. The necessity for having mechanisms of host defense to pathogens often turns nonpathogen signaling into a maladaptive mechanism for damage and scarring in renal tissue. This happens because intrarenal inflammation is generally unable to attenuate the metabolic, hemodynamic, toxic, or autoimmune drivers of kidney injury. These insights should help us to define the genetic risks for immunopathology as factors in renal progression. Blocking nonpathogen activation of the innate immune system is likely to be an important therapeutic strategy

for attenuating this ancillary renal inflammation.

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DISCLOSURES

None.

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